

Award Number: W81XWH-14-1-0077

TITLE: A NOVEL THERAPEUTIC FOR THE TREATMENT AND PREVENTION OF HEARING LOSS
FROM ACOUSTIC TRAUMA

PRINCIPAL INVESTIGATOR: DR. STEVEN HENRY HEFENEIDER

CONTRACTING ORGANIZATION: 13THERAPEUTICS, INC.
Portland, OR 97201

REPORT DATE: May 2015

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.

1. REPORT DATE XXXXXXX May 2015		2. REPORT TYPE Annual		3. DATES COVERED 05/01/2014-04/30/2015	
4. TITLE AND SUBTITLE A Novel Therapeutic for the Treatment and Prevention of Hearing Loss from Acoustic Trauma				5a. CONTRACT NUMBER : W81XW-14-1-0077	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Steven Hefeneider; Sharon McCoy E-Mail: steve@13therapeutics.com ; sharon@13therapeutics.com				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) 13therapeutics, Inc., 4640 SW Macadam Ave., Suite 200D, Portland, OR 97239				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT The most significant finding during this research period was that our therapeutic peptide, T1/P13, was efficacious in treating permanent and severe noise induced hearing loss. Using a steady-state model of noise exposure (117 db for 2 hours), T1/P13 was administered one hour after noise using a variety of doses and routes of administration. Both topical (ear drop) and subcutaneous (SQ) routes of administration of T1/P13 resulted in a reduction in hearing loss seen after noise insult, with the most dramatic improvement seen after SQ administration.					
15. SUBJECT TERMS Noise-induced hearing loss; Toll-like receptor (TLR), Auditory brainstem response (ABR); Intra-cellular signaling pathways; tympanic membrane					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	10	19b. TELEPHONE NUMBER (include area code)

Table of Contents

	<u>Page</u>
1. Introduction.....	1
2. Keywords.....	1
3. Accomplishments.....	1
4. Impact.....	4
5. Changes/Problems.....	4
6. Products.....	5
7. Participants & Other Collaborating Organizations.....	5
8. Special Reporting Requirements.....	6
9. Appendices.....	6

1. INTRODUCTION

Hearing loss caused by noise exposure is a significant medical problem, with work-place associated noises such as machinery a frequent cause of hearing loss in both the civilian and military populations. In the military population, acute noise exposure resulting from explosions, blasts, or gunshots, represents an additional risk for hearing loss. Currently there is no treatment for hearing loss caused by noise exposure, and prevention is focused on reducing noise exposure and utilization of hearing protection devices. Recent investigations have identified at least three signaling pathways as being critical in contributing to cochlear hair cell damage or death by apoptosis following noise exposure. Interconnected with these pathways is the Toll-like receptor (TLR) signaling pathway. Our laboratory has recently identified and characterized a novel peptide inhibitor of TLR signaling termed P13. The global hypothesis of this application is that modulation of TLR signaling by P13 will prevent or limit activation of these interconnected signaling pathways that trigger the damage or apoptotic death of cochlear hair cells. Inhibition of these pathways by P13 will prevent/limit cochlear hair cell damage/death following noise exposure, leading to improved hearing.

2. KEYWORDS

Noise-induced hearing loss
Toll-like receptor (TLR)
MAPK/JNK pathway
Reactive oxygen species
Auditory Brainstem Response (ABR)
Intra-cellular signaling pathways
Apoptosis
Cochlear hair cell death
Tympanic membrane

3. ACCOMPLISHMENTS

➤ What were the major goals of the project?

- A) Determine the preclinical efficacy of P13, when administered after acoustic trauma as a treatment protocol, to limit hearing impairment.
- B) Establish the kinetics of P13 peptide transfer across the tympanic membrane following topical (ear drop) administration and quantify the amount of P13 that reaches the inner ear after acoustic trauma.
- C) Determine the preclinical efficacy of P13, when administered prior to acoustic trauma as a preventative protocol, to limit hearing impairment
- D) Determine the mechanism of action by which P13 limits hearing impairment in models of acoustic trauma

➤ What was accomplished under these goals?

During this project period, we have collected data to complete Aim #1, "Determine the preclinical efficacy of P13, when administered after acoustic trauma as a treatment protocol, to limit hearing impairment" and Aim #2 "Establish the kinetics of P13 peptide transfer across the tympanic membrane following topical (ear drop) administration and quantify the amount of P13 that reaches the inner ear after acoustic trauma".

- A) A Research Assistance with several years of experience in conducting noise exposure and ABR experiments was hired.
- B) Experiments were conducted to ensure that the noise-exposure chamber for steady state exposure and the ABR machine were functioning correctly.
- C) Experiments were conducted to finalize the noise-insult parameters in the murine steady state model. Two noise insult levels, 110 db, which we have used in our previous studies, and 117 db, which has been used in other experimental labs were tested. The 110 db model demonstrated hearing loss at 16 and 32 kHz, but not at 4 or 8 kHz, while the 117 db model demonstrated hearing loss at all four frequencies, and the degree of hearing loss was greater at 16 and 32 kHz than hearing loss seen in the 110 db model at those frequencies. Based on these studies, we have elected to proceed with 117 db noise exposure.
- D) Timing studies were completed in the murine steady state model of noise-induced hearing loss using 117 db noise insult to determine how long the hearing loss due to noise exposure remained in effect. ABRs were completed 1 day, 1 week and 6-8 weeks post noise insult. For all frequencies tested (4, 8, 16 and 32 kHz), the hearing thresholds were approximately 10 db lower (better hearing) at one week as compared to one day post noise exposure, indicating some degree of temporary hearing loss. The hearing thresholds measured at 6-8 weeks post noise were approximately the same as one week post-noise at all frequencies (permanent hearing loss). The permanent hearing loss (seen at 1-8 weeks) was substantial and ranged from 25 db (8 kHz) to 40 db (16 kHz).
- E) Experiments were conducted to determine which P13 derivative to use for these studies. The anti-inflammatory peptide proposed in this grant, P13, has multiple derivatives. The best derivative, P13/T1 was compared directly to P13 in the noise-induced hearing loss murine steady-state model using 117 db noise insult, and demonstrated greater efficacy at reducing hearing loss due to noise. Based on these studies, we have elected to proceed with P13/T1 for the remainder of the experiments.
- F) Dosing studies were completed using P13/T1 in the murine steady state model of noise-induced hearing loss using 117 db noise insult, and administering P13/T1 as ear drops. We tested three doses of P13/T1, 10 µg, 50 µg, and 100 µg, administered topically as ear drops one hour post noise insult. ABRs were completed at one week and 6-8 weeks post noise insult. For controls we used both PBS and a control peptide. The control peptide and PBS were not significantly different from each other at any frequency. Both the 10 µg and 50 µg doses were effective at reducing the hearing loss seen after noise insult as compared to a control peptide (ANOVA across frequencies for 10 µg $p=0.0012$, for 50 µg $p=0.0452$). The best improvement in hearing was 20 db at 4 kHz following the 10 µg dose. These results were the same at both one week and 6-8 weeks.
- G) We then tested three additional routes of administration for P13/T1, subcutaneous (SQ), trans-tympanic injection, and retro-orbital injection. The purpose of these studies was to both verify the peptide effect and to determine if a different route of peptide administration would be more efficacious than ear drop administration.
 - i) For the subcutaneous studies, we examined a dose response. Either 100, 500 or 1000 µg P13/T1 was administered SQ 1 hour post noise insult in the 117 db noise insult steady-state model and compared to PBS injected animals. 100 and 500 µg P13/T1 demonstrated a reduction in the hearing loss induced by noise. (ANOVA for 100 µg

p=0.0106 and for 500 µg p=0.0001). The 500 µg dose given SQ demonstrated the best hearing improvement seen to date, by any route of administration.

ii) P13/T1 was examined using trans-tympanic injections. The injection itself resulted in an inflammatory response, which negated any potential effect on hearing. We speculate this may be due to the anatomy of the mouse ear, as in guinea pigs this administration route has demonstrated efficacy.

iii) Both P13 and P13/T1 was examined using direct IV injections, administered retro-orbitally. No effect on hearing thresholds was noted using this route of administration.

H) We examined multiple administrations of P13/T1 in the steady-state model, both by ear drop and SQ injections.

i) Two ear drop administrations (10 µg each) given 10 minutes apart, one hour after noise insult were examined. ABRs were completed one week post noise insult. Giving two doses of 10 µg, 10 minutes apart, demonstrated an equal, but not superior, effect on ABR thresholds as compared to one dose.

ii) Two ear drop administrations (one group 10 µg each dose and one group 50 µg each dose) were given 24 hours apart, with the first dose given one hour after noise insult. No impact on hearing thresholds were seen with this administration protocol.

iii) Two SQ doses were given (one group 100 µg each dose and one group 500 µg each dose) were given 24 hours apart, with the first dose given one hour after noise insult. Although there was some impact on hearing thresholds, the effect was not as great as a single SQ dose.

I) To complete Specific Aim #2 “Establish the kinetics of P13 peptide transfer across the tympanic membrane following topical (ear drop) administration and quantify the amount of P13 that reaches the inner ear after acoustic trauma”, a method of detecting and quantifying T1/P13 in tissue must be developed. We have begun method development to use mass spectrometry to detect P13 in tissue. Preliminary experiments have been completed and we are able to detect T1/P13 in sample tissue. Further experiments will use a stable label internal standard for quantification.

J) Two noise exposure models are described in this grant, the steady-state model, and the impulse model. During this granting period, the impulse noise chamber was designed, constructed and validated by sound engineers.

➤ **What opportunities for training and professional development has the project provided?**

Nothing to report

➤ **How were the results disseminated to communities of interest?**

Nothing to report

➤ **What do you plan to do during the next reporting period to accomplish these goals?**

During this reporting period we have completed data collection on all aspects of the steady state model except for examining the length of time we can treat post-noise and retain efficacy. The experiments to date have demonstrated efficacy when treatment is administered one hour post noise exposure. For the next reporting period we will determine the treatment window in the steady-state model for the administration of P13/T1 after noise exposure. We will begin with six hours post noise, and extend the window as appropriate. Once this is established, we will begin the mechanism studies by examining the gene expression profile with and without P13/T1 after noise exposure. We will determine the efficacy of P13/T1 as a preventative protocol to limit hearing impairment and also begin the impulse-model experiments.

4. IMPACT

➤ **What was the impact on the development of the principal discipline(s) of the project?**

The data collected during this reporting period adds to the body of knowledge surrounding the mechanism and treatment of noise-induced hearing loss. By demonstrating efficacy in reducing hearing loss due to noise exposure using a TLR inhibitor, we demonstrate that the toll-like receptor signaling pathway plays a role in noise-induced hearing loss, and that a TLR pathway inhibitor may be a valuable therapeutic in treating hearing loss due to noise exposure.

➤ **What was the impact on other disciplines?**

Nothing to report

➤ **What was the impact on technology transfer?**

Nothing to report

➤ **What was the impact on society beyond science and technology?**

Nothing to report

5. CHANGES/PROBLEMS:

➤ **Changes in approach and reasons for change**

Nothing to report

➤ **Actual or anticipated problems or delays and actions or plans to resolve them**

Nothing to report

➤ **Changes that had a significant impact on expenditures**

Nothing to report

➤ **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

Nothing to report

6. PRODUCTS

➤ **Publications, conference papers, and presentations**

Nothing to report

➤ **Websites or other Internet sites**

Nothing to report

➤ **Technologies or techniques**

Nothing to report

➤ **Inventions, patent applications, and/or licenses**

Nothing to report

➤ **Other products**

Nothing to report

7. PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS

➤ **What individuals have worked on the project?**

Name:	Steven Hefeneider, Ph.D.
Project Role:	Principal Investigator
Nearest person month worked:	6 months
Contribution to Project:	Dr. Hefeneider has overseen all phases of the project to date, including experimental design and result interpretation.

Name:	Sharon McCoy
Project Role:	Collaborator
Nearest person month worked:	5 months
Contribution to Project:	Ms. McCoy has been involved with all phases of the project to date, including experimental design and data analysis

Name:	Beth Kempton
Project Role:	Research Associate
Nearest person month worked:	6 months
Contribution to Project:	Ms. Kempton has performed all the noise induction and ABR studies to date

➤ **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Nothing to report

➤ **What other organization were involved as partners?**

Organization Name: Oregon Health and Science University

Location of Organization: Portland, Oregon

Partner's contribution to the project: 13therapeutics has subcontracted with Oregon Health and Science University to conduct the noise exposure studies and perform the Auditory Brainstem Response testing.

8. SPECIAL REPORTING REQUIREMENTS

The updated Quad Chart is submitted as an appendix.

9. APPENDICES

Quad Chart

A Novel Therapeutic for the Treatment and Prevention of Hearing Loss from Acoustic Trauma

Report Period 05/01/2014-04/30/2015
W81XW-14-1-0077



PI: Steven H. Hefeneider, Ph.D.

Org: 13therapeutics, Inc.

Award Amount: \$1,046,655.00

Study/Product Aim(s)

- 1) Determine the preclinical efficacy of P13, when administered after acoustic trauma as a treatment protocol, to limit hearing impairment.
- 2) Establish the kinetics of P13 peptide transfer across the tympanic membrane following topical (ear drop) administration and quantify the amount of P13 that reaches the inner ear after acoustic trauma.
- 3) Determine the preclinical efficacy of P13, when administered prior to acoustic trauma as a preventative protocol, to limit hearing impairment
- 4) Determine the mechanism of action by which P13 limits hearing impairment in models of acoustic trauma

Exposure to Different Noise Sources



Steady-state



Impulse

Treatment with P13



Audiometric and Histological Readouts

Accomplishment: Data collected for Specific Aim #1 and #2.

Timeline and Cost

Activities	CY	14-15	15-16
Determine the efficacy of P13, as a treatment protocol, to limit hearing impairment.			
Establish the kinetics of P13 peptide transfer across the tympanic membrane and quantify the amount of P13 that reaches the inner ear after acoustic trauma.			
Determine the preclinical efficacy of P13 as a preventative protocol, to limit hearing impairment			
Determine the mechanism of action by which P13 limits hearing impairment in models of acoustic trauma			
Estimated Budget		\$472,477	\$574,178

Updated: 05/22/2015

Goals/Milestones

CY14-15 Goal – 1) Determine the efficacy of P13, as a treatment protocol, to limit hearing impairment and 2) Establish the kinetics of P13 peptide transfer across the tympanic membrane and quantify the amount of P13 that reaches the inner ear after acoustic trauma.

- ✓ Completed model development, dosing (single and multiple) and route of administration studies for P13/T1 administered one hour post noise exposure in the steady-state model
- ✓ Began model development to use mass spectrometry to detect P13/T1 in tissue
- ✓ Completed the design, construction and validation of the impulse noise chamber

CY15-16 Goals – 1) Complete the impulse model studies, 2) Complete the kinetic studies 3) Determine the preclinical efficacy of P13/T1 as a preventative protocol, to limit hearing impairment and 4) Determine the mechanism of action by which P13/T1 limits hearing impairment in models of acoustic trauma

Budget Expenditure to Date

Projected Expenditure: \$360,000.00

Actual Expenditure: \$333,993.28